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Luffa acutangula Roxb. Tea Promotes Developmental Toxicity to Rats

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Abstract: The tea made from *Luffa acutangula* fruits has been largely used by women for abortion induction and abortions occurred in ruminants that had ingested the fruits. The research aimed to study the abortive effect of *L. acutangula* tea in rats. Eleven pregnant Wistar female rats were used. On the 15th gestational day, six rats were dosed with 10 mL kg^{-1} of *L. acutangula* tea (50 g of dried fruit in 100 mL of water) and the other five rats were dosed with saline solution. On the 21st gestational day, all the rats were submitted to cesarean section. There was no difference between the two groups on body weight and body weight gain and no sign of maternal toxicity was observed in females throughout the experiment. The number of points of implantation, live and dead fetuses, corpora lutea and points of reabsorption did not differ between groups but it was found reduced fetal weight in the group treated with *L. acutangula*. The search for external malformations revealed a fetus with cleft palate from a plant-treated mother. No lesion was found at histological evaluation of placentas. In conclusion, the ingestion of *L. acutangula* during pregnancy may promote developmental toxicity.

Key words: Reproductive toxicology, malformation, cleft palate, abortion, fruit, tea

INTRODUCTION

Plants of the Cucurbitaceae family are characteristically but not exclusively, producers of cucurbitacins, highly oxygenated triterpenes. Cucurbitacins isolated from plants of the genus *Luffa* have various pharmacological properties including anti-inflammatory, antimicrobial, antitumoral and hepatocurative effects and they are cytotoxic (Miró, 1995). Other compounds isolated from *Luffa* species include luffaculins, which are ribosome-inactivating proteins (Ng *et al*., 1992; Chan *et al*., 1994; Lin *et al*., 2002; Wang and Ng, 2002; Hou *et al*,. 2007), the trypsin inhibitors LA-1 and LA-2 (Haldar *et al*., 1996) and a lectin that is specific for chito-oligosaccharides (Anantharam *et al*., 1986).

The tea made from fruits of *Luffa acutangula* fruits has been widely used by women for induction of abortion and as a purgative (Lorenzi and Matos, 2002). Furthermore, several farmers from the northeastern region of Brazil have reported abortions in ruminants that had ingested fruits of this plant (Da Silva *et al*., 2006). However, studies of the interference of this plant with reproduction are needed. The research aimed to study the abortifacient effect of *L. acutangula* tea in rats.

MATERIALS AND METHODS

Mature fruits of *L. acutangula* Roxb. were collected at Catole do Rocha municipality, Paraíba state, Brazil. Botanical identification was done by Prof. Odaci Fernandes de Olivera, a retired professor from Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, RN, Brazil. A total of 50 g of dried powdered fruits were added to 100 mL of distilled water. The mixture was warmed until it began boiling and it was then filtered. The solution obtained was kept refrigerated for up to 7 days.

Adult male and virgin female Wistar rats were obtained at the age of about 12 weeks from the Department of Animal Sciences, Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil. Food and tap water were provided *ad libitum*. For mating, two females were placed together with one male. Females that showed evidence of mating (a vaginal plug, or a vaginal smear showing sperm cells) were assigned in rotation to each dose group until the required 12 females had been allotted to each group. The day on which there was evidence of mating was recorded as day 0 of gestation. During gestation, the female rats were housed individually in plastic cages measuring 40×50×20 cm, which were

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covered with metal lids. The animal room was maintained at 22-24°C and on a 12-h light/dark cycle. Eleven pregnant Wistar female rats were used for the experiment. On the 15th day of gestation, six rats were dosed by gavage with 10 mL kg^{-1} of the infusion of *L. acutangula* and the other five rats were dosed with 10 mL kg^{-1} of saline solution. On the 21st day of gestation, all the rats were submitted to Cesarean section.

The number of points of implantation, live and dead fetuses, corpora lutea and reabsorption points was recorded. Fetuses and placentas were weighed individually and the fetuses were evaluated carefully for the presence of external malformations. The placentas were collected for pathological study. Paraffin-embedded sections were stained with Hematoxylin and Eosin (H and E).

Data are reported as the mean±standard deviation and they were compared using the unpaired t-test for parametric data or the Mann-Whitney test for non-parametric data using GraphPad Prism v. 4 for Mac. The level of significance was set at $p<0.05$.

RESULTS

The body weight and gains in body weight of rats dosed with *L. acutangula* did not show significant ($p>0.05$) differences from control rats (Table 1). Furthermore, no clinical signs of maternal toxicity were observed.

The data on reproduction are presented in Table 2. The number of points of implantation, live and dead fetuses, corpora lutea and points of reabsorption did not differ ($p>0.05$) between groups but reduced ($p<0.05$) fetal weight was found in the group treated with *L. acutangula*.

Table 1: Body weight and gain in body weight (in g) of pregnant rats dosed with *Luffa acutangula* or saline solution (control) on the 15th day of pregnancy. The data are presented as means followed by the respective standard deviation

Body weight	Days	Control	Treated
Total	1	184.8±17.00	182.8±7.19
	15	244.5±19.40	245.0±13.6
	21	303.0± 14.4	300.7±27.6
Weight gain	1-15	59.8±7.180	62.2±19.1
	15-21	53.8±10.20	62.8±17.6

Table 2: Reproductive performance (mean±SD) of pregnant rats dosed with *Luffa acutangula* or saline solution (control) on the 15th day of pregnancy

Variables	Control	Treated
Number of corpora lutea	11.2±1.920	11.5±0.840
Number of implantations	10.4±1.820	10.7±1.370
Number of live fetuses	10.0±2.550	8.17±2.04
Pre-implantation loss (%)	6.82±7.03	7.20±9.79
Post-implantation loss (%)	5.00±11.2	21.5±23.50
Fetal weight (g)	3.72±0.22	3.22±0.38*
Placental weight (g)	0.46±0.04	0.51±0.10*

*p<0.05 (unpaired t test)

Fig. 1: Heads from fetuses of pregnant rats dosed with *Luffa acutangula* (above) or saline solution (below) at 15 days of pregnancy. Note presence of cleft palate in fetus from treated rat

The search for external malformations revealed a fetus with a cleft palate from a female rat treated with *L. acutangula* (Fig. 1). The histological evaluation of the placentas showed no lesions on treated and control rats.

DISCUSSION

Poisonous plants often affect animal reproduction, causing dysfunction including abortions, infertility and teratogenesis. In Brazil, poisonous plants that promote abortions and neonatal loss include *Aspidosperma pyrifolium* Mart. (De Souza Lima and Soto-Blanco, 2010), *Ateleia glazioviana* Baill. (Stolf *et al*., 1994), *Tetrapterys acutifolia* Cav., *Tetrapterys multiglandulosa* A. Juss. (Tokarnia *et al*., 1989, 1998) and *Stryphnodendron obovatum* Benth. Several other species occasionally promote abortions, for example *Amaranthus spinosus* L. (Peixoto *et al*., 2003) and cyanogenic plants (Soto-Blanco and Gorniak, 2004).

The treatment with *L. acutangula* did not affect maternal body weight and no clinical signs of maternal toxicity were observed. Thus, no evident toxicity was produced in dams. It is well known that maternal toxicity is responsible for disturbances of fetal development because maternal-fetal interactions are affected (Khera, 1984; Chernoff *et al*., 1989). In the study, any possible interference in fetal development from maternal toxicity may be considered improbable. In the study, the fetal weight was reduced in the group treated with *L. acutangula* but other reproductive data did not show significant variation. The reduced fetal weight is a

significant effect because this weight is one of the most important parameters measured in studies of developmental toxicity (Christian, 2001). Thus, it can be stated that *L. acutangula* presents a fetotoxic effect.

Luffa acutangula is popularly used by women who aim to interrupt gestation (Lorenzi and Matos, 2002). Furthermore, several farmers from the northeastern region of Brazil have reported abortions in ruminants that had ingested fruits of *L. acutangula* (Da Silva *et al*., 2006). Several studies have identified proteins from *L. acutangula* and other *Luffa* species that have abortifacient action (Schilling and Heiser, 1981; Ng *et al*., 1992; Chan *et al*., 1994; Lin *et al*., 2002; Wang and Ng, 2002) including the proteins luffin b and lufaculin (Chan *et al*., 1994). These proteins promote the inactivation of ribosomes (Ng *et al*., 1992; Chan *et al*., 1994; Wang and Ng, 2002; Hou *et al*., 2007), which affects the synthesis of cellular proteins and consequently, embryogenesis and fetal development. In the study, the post-implantation loss in rats dosed with *L. acutangula* was 21.5%, which is much higher than that of the control group (5%) but this result did not reach statistical significance.

The search for external malformations revealed a fetus with a cleft palate from a female rat treated with *L. acutangula*. To the knowledge, this is the first time that a malformation has been attributed to *L. acutangula*. However, it has been reported that *Luffa operculata* was responsible for significant alterations in the palatal epithelium of the frog (Menon-Miyake *et al*., 2005). Several chemicals are known to induce cleft palate, including the non-steroidal anti-inflammatory drug acetylsalicylic acid (DePass and Weaver, 1982), the corticosteroid dexamethasone (Bonner, 1984), the solvent xylene (Hood and Ottley, 1985), the anticonvulsant sodium valproate (Alsdorf and Wyszynski, 2005) and the environmental contaminant dioxin (Bock and Köhle, 2006).

The placenta is an important organ that performs a number of different and specialized functions in pregnancy. Several chemicals may promote damage to the placenta and the damaged placenta may affect embryonic and fetal development (Goodman *et al*., 1982; Soto-Blanco *et al*., 2009). In the research, no lesions were found on histological evaluation of the placentas of treated rats. Thus, the toxic effects of *L. acutangula* on the fetuses could not be secondary to a damaged placenta.

CONCLUSION

The ingestion of *L. acutangula* during pregnancy may promote developmental toxicity. In rats, this was shown by reduced fetal weight and the occurrence of cleft palate.

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